



RECEIVED

APR 26 2001

## SEQUENCE LISTING

TECH CENTER 1600/2900

<110> Chemicon International, Inc.  
LENG, Jay

<120> PROTEASE SPECIFIC CLEAVABLE LUCIFERASES AND METHODS OF USE THEREOF

<130> CHEM1110

<140> US 09/619,047

<141> 2000-07-18

<160> 29

<170> PatentIn version 3.0

<210> 1

<211> 936

<212> DNA

<213> Renilla reniformis

<220>

<221> CDS

<222> (1) .. (936)

<400> 1

atg act tcg aaa gtt tat gat cca gaa caa agg aaa cgg atg ata act 48  
Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
1 5 10 15

ggt ccg cag tgg tgg gcc aga tgt aaa caa atg aat gtt ctt gat tca 96  
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
20 25 30

ttt att aat tat tat gat tca gaa aaa cat gca gaa aat gct gtt att 144  
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
35 40 45

ttt tta cat ggt aac gcg gcc tct tct tat tta tgg cga cat gtt gtg 192  
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val  
50 55 60

cca cat att gag cca gta gcg cgg tgt att ata cca gat ctt att ggt 240  
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
65 70 75 80

atg ggc aaa tca ggc aaa tct ggt aat ggt tct tat agg tta ctt gat 288  
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
85 90 95

cat tac aaa tat ctt act gca tgg ttt gaa ctt ctt aat tta cca aag 336  
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
100 105 110

aag atc att ttt gtc ggc cat gat tgg ggt gct tgt ttg gca ttt cat 384  
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His  
115 120 125

tat agc tat gag cat caa gat aag atc aaa gca ata gtt cac gct gaa 432  
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu  
130 135 140

agt gta gta gat gtg att gaa tca tgg gat gaa tgg cct gat att gaa 480  
 Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
 145 150 155 160

gaa gat att gcg ttg atc aaa tct gaa gaa gga gaa aaa atg gtt ttg 528  
 Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
 165 170 175

gag aat aac ttc ttc gtg gaa acc atg ttg cca tca aaa atc atg aga 576  
 Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg  
 180 185 190

aag tta gaa cca gaa gaa ttt gca gca tat ctt gaa cca ttc aaa gag 624  
 Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
 195 200 205

aaa ggt gaa gtt cgt cgt cca aca tta tca tgg cct cgt gaa atc ccg 672  
 Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
 210 215 220

tta gta aaa ggt ggt aaa cct gac gtt gta caa att gtt agg aat tat 720  
 Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
 225 230 235 240

aat gct tat cta cgt gca agt gat gat tta cca aaa atg ttt att gaa 768  
 Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
 245 250 255

tcg gat cca gga ttc ttt tcc aat gct att gtt gaa ggc gcc aag aag 816  
 Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
 260 265 270

ttt cct aat act gaa ttt gtc aaa gta aaa ggt ctt cat ttt tcg caa 864  
 Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln  
 275 280 285

gaa gat gca cct gat gaa atg gga aaa tat atc aaa tcg ttc gtt gag 912  
 Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
 290 295 300

cga gtt ctc aaa aat gaa caa taa 936  
 Arg Val Leu Lys Asn Glu Gln  
 305 310

<210> 2

<211> 311

<212> PRT

<213> Renilla reniformis

<400> 2

Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
 1 5 10 15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
 20 25 30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
 35 40 45

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val  
 50 55 60

Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly  
 65 70 75 80

Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp  
 85 90 95

His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys  
 100 105 110

Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His  
 115 120 125

Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu  
 130 135 140

Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu  
 145 150 155 160

Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
 165 170 175

Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg  
 180 185 190

Lys Leu Glu Pro Glu Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu  
 195 200 205

Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
 210 215 220

Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
 225 230 235 240

Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
 245 250 255

Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
 260 265 270

Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln

275	280	285	
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu			
290	295	300	
Arg Val Leu Lys Asn Glu Gln			
305	310		
<210> 3			
<211> 936			
<212> DNA			
<213> Renilla reniformis (mutated sequence)			
<220>			
<221> CDS			
<222> (1)..(936)			
<400> 3			
atg act tcg aaa gtt tat gat cca gaa caa agg aaa cgg atg ata act			48
Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr			
1	5	10	15
ggt ccg cag tgg tgg gcc aga tgt aaa caa atg aat gtt ctt gat tca			96
Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser			
	20	25	30
ttt att aat tat tat gat tca gaa aaa cat gca gaa aat gct gtt att			144
Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile			
	35	40	45
ttt tta cat ggt aac gcg gcc tct tct tat tta tgg cga cat gtt gtg			192
Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val			
	50	55	60
cca cat att gag cca gta gcg cgg tgt att ata cca gat ctt att ggt			240
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly			
65	70	75	80
atg ggc aaa tca ggc aaa tct ggt aat ggt tct tat agg tta ctt gat			288
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp			
	85	90	95
cat tac aaa tat ctt act gca tgg ttt gaa ctt ctt aat tta cca aag			336
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys			
	100	105	110
aag atc att ttt gtc ggc cat gat tgg ggt gct tgt ttg gca ttt cat			384
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His			
	115	120	125
tat agc tat gag cat caa gat aag atc aaa gca ata gtt cac gct gaa			432
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu			
	130	135	140
agt gta gta gat gtg att gaa tca tgg gat gaa tgg cct gat att gaa			480
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu			
145	150	155	160

gaa gat att gcg ttg atc aaa tct gaa gaa gga gaa aaa atg gtt ttg 528  
 Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu  
                   165                  170                  175

gag aat aac ttc ttc gtg gaa acc atg ttg cca tca aaa atc atg aga 576  
 Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg  
                   180                  185                  190

aag tta gaa cca gac gaa gtt gac gca tat ctt gaa cca ttc aaa gag 624  
 Lys Leu Glu Pro Asp Glu Val Asp Ala Tyr Leu Glu Pro Phe Lys Glu  
                   195                  200                  205

aaa ggt gaa gtt cgt cgt cca aca tta tca tgg cct cgt gaa atc ccg 672  
 Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro  
                   210                  215                  220

tta gta aaa ggt ggt aaa cct gac gtt gta caa att gtt agg aat tat 720  
 Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr  
                   225                  230                  235                  240

aat gct tat cta cgt gca agt gat gat tta cca aaa atg ttt att gaa 768  
 Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu  
                   245                  250                  255

tcg gat cca gga ttc ttt tcc aat gct att gtt gaa ggc gcc aag aag 816  
 Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys  
                   260                  265                  270

ttt cct aat act gaa ttt gtc aaa gta aaa ggt ctt cat ttt tcg caa 864  
 Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln  
                   275                  280                  285

gaa gat gca cct gat gaa atg gga aaa tat atc aaa tcg ttc gtt gag 912  
 Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu  
                   290                  295                  300

cga gtt ctc aaa aat gaa caa taa 936  
 Arg Val Leu Lys Asn Glu Gln  
                   305                  310

<210> 4  
 <211> 311  
 <212> PRT  
 <213> Renilla reniformis (mutated sequence)

<400> 4

Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr  
 1                  5                  10                  15

Gly Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser  
                   20                  25                  30

Phe Ile Asn Tyr Tyr Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile  
                   35                  40                  45

Phe Leu His Gly Asn Ala Ala Ser Ser Tyr Leu Trp Arg His Val Val

50		55		60
Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp Leu Ile Gly				
65		70		75 80
Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp				
	85		90	95
His Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys				
	100		105	110
Lys Ile Ile Phe Val Gly His Asp Trp Gly Ala Cys Leu Ala Phe His				
	115		120	125
Tyr Ser Tyr Glu His Gln Asp Lys Ile Lys Ala Ile Val His Ala Glu				
	130		135	140
Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro Asp Ile Glu				
145		150	155	160
Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu				
	165		170	175
Glu Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg				
	180		185	190
Lys Leu Glu Pro Asp Glu Val Asp Ala Tyr Leu Glu Pro Phe Lys Glu				
	195		200	205
Lys Gly Glu Val Arg Arg Pro Thr Leu Ser Trp Pro Arg Glu Ile Pro				
	210		215	220
Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val Arg Asn Tyr				
225		230	235	240
Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu				
	245		250	255
Ser Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys				
	260		265	270
Phe Pro Asn Thr Glu Phe Val Lys Val Lys Gly Leu His Phe Ser Gln				
	275		280	285
Glu Asp Ala Pro Asp Glu Met Gly Lys Tyr Ile Lys Ser Phe Val Glu				
290		295	300	

Arg Val Leu Lys Asn Glu Gln  
305 310

<210> 5  
<211> 8  
<212> PRT  
<213> Artificial

<220>  
<223> Description of Artificial Sequence: Protease recognition sequence  
  
<400> 5

Ser Gln Asn Tyr Pro Ile Val Gln  
1 5

<210> 6  
<211> 10  
<212> PRT  
<213> Artificial

<220>  
<223> Description of Artificial Sequence: Protease recognition sequence  
  
<400> 6

Lys Ala Arg Val Leu Ala Glu Ala Met Ser  
1 5 10

<210> 7  
<211> 10  
<212> PRT  
<213> Artificial

<220>  
<223> Description of Artificial Sequence: Protease recognition sequence  
  
<400> 7

Pro Ser Pro Arg Glu Gly Lys Arg Ser Tyr  
1 5 10

<210> 8  
<211> 5  
<212> PRT  
<213> Artificial

<220>  
<223> Description of Artificial Sequence: Protease recognition sequence  
  
<400> 8

Tyr Val Ala Asp Gly  
1 5

<210> 9  
<211> 8  
<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 9

Met Phe Gly Gly Ala Lys Lys Arg  
1 5

<210> 10

<211> 10

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 10

Gly Val Val Asn Ala Ser Ser Arg Leu Ala  
1 5 10

<210> 11

<211> 9

<212> PRT

<213> ARTIFICIAL

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 11

Leu Ile Ala Tyr Leu Lys Lys Ala Thr  
1 5

<210> 12

<211> 7

<212> PRT

<213> ARTIFICIAL

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 12

Val Lys Met Asp Ala Glu Phe  
1 5

<210> 13

<211> 17

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 13

Phe Leu Ala Glu Gly Gly Gly Val Arg Gly Pro Arg Val Val Glu Arg  
1 5 10 15



His

<210> 14  
 <211> 13  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 14

Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His  
 1 5 10

<210> 15  
 <211> 8  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 15

Lys Pro Ala Leu Phe Phe Arg Leu  
 1 5

<210> 16  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 16

Ile Glu Pro Asp  
 1

<210> 17  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 17

Asp Glu Thr Asp  
 1

<210> 18  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 18

Trp Glu His Asp  
 1

<210> 19  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 19

Tyr Val Ala Asp  
 1

<210> 20  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 20

Asp Glu His Asp  
 1

<210> 21  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<400> 21

Asp Glu Val Asp  
 1

<210> 22  
 <211> 4  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Description of Artificial Sequence: Protease recognition sequence

<220>  
 <221> VARIANT  
 <222> (1)..(1)  
 <223> Xaa is Trp or Ley

<400> 22

Xaa Glu His Asp  
1

<210> 23

<211> 4

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<220>

<221> VARIANT

<222> (3)..(3)

<223> Xaa is Ile or His

<400> 23

Val Glu Xaa Asp  
1

<210> 24

<211> 4

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 24

Leu Glu Thr Asp  
1

<210> 25

<211> 4

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 25

Leu Glu His Asp  
1

<210> 26

<211> 4

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<220>

<221> VARIANT

<222> (1)..(3)

<223> Xaa is any amino acid

<400> 26

Xaa Xaa Xaa Asp  
1

<210> 27

<211> 8

<212> PRT

<213> Artificial

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 27

Arg Pro Leu Gly Ile Ile Gly Gly  
1 5

<210> 28

<211> 3

<212> PRT

<213> ARTIFICIAL

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 28

Glu Gly Arg  
1

<210> 29

<211> 3

<212> PRT

<213> ARTIFICIAL

<220>

<223> Description of Artificial Sequence: Protease recognition sequence

<400> 29

Val Leu Lys  
1

cont  
2

Sub  
B1  
cont